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Population dynamics of *Aspergillus flavus* in the air of an intensively cultivated region of south-west Arizona

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Airborne propagules of *Aspergillus flavus* were quantified to investigate population dynamics of *A. flavus* in a region of south-west Arizona prone to epidemics of aflatoxin contamination of cottonseed. Air was sampled continuously from May 1997 to March 1999 at two sites using Burkard cyclone samplers. One sampler was initially at the centre of 65 ha of cotton treated with an atoxigenic strain of *A. flavus* to manage aflatoxin contamination of cottonseed. The second sampler was 0·6 km from the treated field. Total fungal colony-forming units (CFU) sampled ranged from 17 to 667 and from 9 to 1277 m^{-3} at the untreated and treated sites, respectively. Counts of *A. flavus* ranged from 0 to 406 m^{-3} and from 0 to 416 per m^{-3} at the untreated and treated sites, respectively. *Aspergillus flavus* comprised 1–46 and 1–51% of the total cultured fungi at the treated and untreated sites, respectively. Peaks in total fungal and *A. flavus* CFU coincided with boll maturation and cotton harvest (days 251–321). Autoregression analysis suggested that there was no difference in total fungal CFU between treated and untreated sites, but the analysis showed that the quantity of *A. flavus* decreased at the treated site. This is probably caused by changes in cropping making the conditions less conducive to growth and reproduction of *A. flavus* in the surrounding fields. The incidence of the S strain of *A. flavus* was highest between May and August. The L strain accounted for up to 100% of the *A. flavus* sampled in the other months, and autoregression analysis showed that the L strain accounted for a greater overall proportion of the *A. flavus* population at the treated site compared with the untreated site. Autoregression analysis also showed the vegetative compatibility group of the applied strain was a greater proportion of L-strain *A. flavus* at the treated site (5–75%) than at the untreated site (0–65%), although this decreased with time. The quantity of *A. flavus* sampled at both treated and untreated sites was correlated with air and soil temperature. Large quantities of *A. flavus* occurred in the soil (up to 34 474 CFU g^{-1}) of cotton fields and on cotton plant parts and debris (up to 272 461 CFU g^{-1}) adjacent to the cyclone samplers. *Aspergillus flavus* is a major constituent of the airborne mycoflora associated with cotton fields in south-west Arizona when temperature is conducive to fungal growth. Although application of atoxigenic *A. flavus* altered the proportion of *A. flavus* strains and vegetative compatibility groups in the aerial mycoflora, the total quantity of *A. flavus* remained similar to that in untreated fields. Dispersal of *A. flavus* between fields suggests that atoxigenic fungi will be most effective in area-wide management programmes.

Keywords: aflatoxin management, agricultural dust, epidemiology, spore sampling

Introduction

Aspergillus flavus can infect cottonseed (*Gossypium indicum*) and other food and feed crops under specific environmental conditions (Diener *et al*., 1987). Concern regarding *A. flavus* infections largely focuses on fungal production of aflatoxins which are potent natural carcinogens. To minimize the risk of toxin carryover to humans or livestock, crop aflatoxin content is strictly regulated (Park *et al*., 1988). *Aspergillus flavus* has been divided into S and L morphotypes, more commonly called strains, based on sclerotial morphology, genetics and physiology (Cotty, 1989). The S strain produces numerous small sclerotia (generally <400 μm in diameter), and on average, much larger concentrations of aflatoxin than the L strain. L-strain isolates produce fewer, larger sclerotia (generally >400 μm in diameter).

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Some isolates of *A. flavus* lack the ability to produce aflatoxins. Application of these atoxigenic strains is used competitively to exclude aflatoxin-producing strains and thereby reduce aflatoxin contamination (Cotty & Bayman, 1993; Cotty, 1994a, Cotty, 2000). Atoxigenic strains of *A. flavus* have effectively reduced contamination in experimental plots of peanut (Dorner *et al*., 1992), corn (Brown *et al*., 1991) and cotton (Cotty, 1994a), and one atoxigenic strain, AF36, is being utilized in a statewide aflatoxin management programme in Arizona. Although aflatoxin contamination is reduced by application of atoxigenic strains (Cotty, 1994a), the overall effect of treatments on quantities of *A. flavus* propagules in the air is unknown. Atoxigenic strain applications seek to competitively exclude aflatoxin producers, and optimal application of the biocontrol agent is dependent on *A. flavus* community dynamics (Cotty *et al*., 1994).

Aspergillus flavus is a common constituent of airborne mycoflora (Morrow *et al*., 1964). Epidemiological studies have defined factors influencing infection of and aflatoxin formation in cottonseed (Ashworth *et al*., 1969a, 1969b; Marsh *et al*., 1973; Lee *et al*., 1986; Diener *et al*., 1987) and the production and dispersal of propagules at specific times in cotton (Lee *et al*., 1986) or in other crops (Bothast *et al*., 1978; Holtmeyer & Wallin, 1980, 1981; Olanya *et al*., 1997). Peaks in quantities of *A. flavus* have been found in the summer months, but the quantity and dynamics of *A. flavus* in the air at different times during the year, and in relation to other fungal propagules in cultivated desert environments, is unknown.

To expand available information on the population dynamics of aerially dispersed propagules of *A. flavus* in south-west Arizona, this study quantified propagules in the air over 2 years at two sites ≈1 km apart. Fields surrounding one site were initially planted with cotton and treated with an atoxigenic strain of *A. flavus*. Fields surrounding the second site were not treated and were initially planted to barley. The objectives of this work were to (i) investigate the year-round population dynamics of *A. flavus* in relation to other fungi in both treated and untreated locations; (ii) assess differences in the dynamics of S and L strains of *A. flavus*; (iii) investigate the effects of treatment with a biocontrol agent on the population structure of *A. flavus*; (iv) explore whether the observed quantities of *A. flavus* could be related to particular environmental factors; and (v) gain insight into optimum times to treat fields with the atoxigenic biocontrol agent.

Materials and methods

Bioaerosol sampling

To monitor the quantity of *A. flavus* propagules in the air, two Burkard Cyclone Samplers (Burkard Manufacturing Co. Ltd, Rickmansworth, UK) were operated continuously from May 1997 to March 1999 (Fig. 1). The samplers were located near Roll, Arizona. One sampler was centred on four 16 ha fields initially planted to cotton treated with an atoxigenic *A. flavus* isolate (strain AF36; ATCC 96045, Cotty & Bayman, 1993) on 2 June 1997. The field (16 ha) immediately south-east of the sampler was treated in May 1996. The second sampler was 0·6 km from the nearest treated field and 1·21 km from the other sampler. During the sampling period various crops were grown in the fields adjacent to the two cyclone samplers (Table 1). At the treated site all four fields surrounding the sampler were initially planted to cotton, followed by wheat, then to lettuce and finally back to wheat. Cotton was present only at the treated site during 1997. All four fields surrounding the sampler at the untreated site were initially planted to barley then rotated to cabbage, cotton and finally to wheat. The two sites had the same crop (wheat) from December 1998 to March 1999. During the 1998 sampling period the untreated site was planted to cotton, although the early cotton season was very cool, resulting in poor crop development and one of the lowest yielding Arizona crops in history.

The biocontrol agent was applied as steam-sterilized wheat seed colonized with the atoxigenic strain, and was spread with a fertilizer drop-box at a rate of 10 kg seed ha^{-1} (Bock & Cotty, 1999). The seed remains on the soil surface, where the biocontrol agent grows and sporulates in the presence of moisture and high temperature. The atoxigenic strain is endemic to the test area and comprised

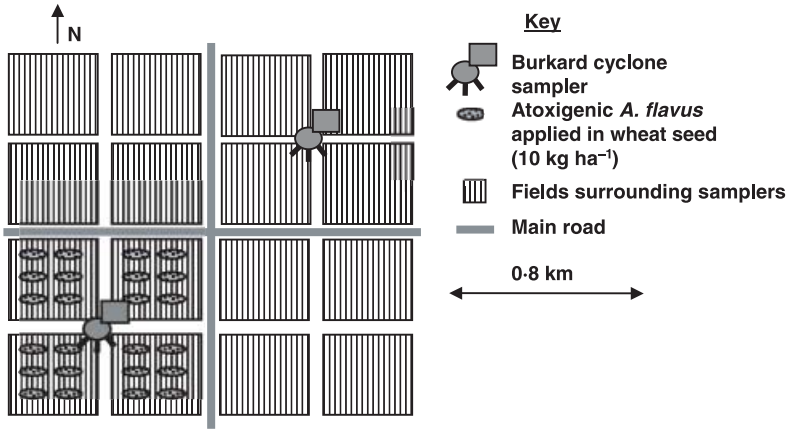

Figure 1 Location of the two Burkard cyclone samplers in relation to fields treated with an atoxigenic vegetative compatibility group of *Aspergillus flavus* in the Mohawk Valley, Arizona.

Table 1 Cropping history[a] of fields adjacent to the Burkard cyclone sampler at treated and untreated sites in the Mohawk Valley, Arizona

	Month											
Field	M	J	J	A	S	O	N	D	J	F	M	A
1997/98												
Treated	ct	ct	ct	ct	ct	ct	fw	wt	wt	wt	wt	wt
Untreated	ba	fw	fw	ca	ca	ca	ca	ca	ca	fw	ct	ct
1998/99												
Treated	wt	wt	fw	lc	lc	lc	lc	wt	wt	wt	wt	
Untreated	ct	ct	ct	ct	ct	ct	fw	wt	wt	wt	wt	

[a]Cropping: ct = cotton, ca = cabbage, lc = lettuce, fw = fallow – soil preparation for next crop, wt = wheat, ba = barley.

1–4% of the *A. flavus* recovered from soils using a culture assay before its application in 1996 (P.J.C., unpublished results).

The Burkard cyclone sampler is ideal for sampling particles in dry environments, and collects a dry dust that allows ready quantification of viable propagules through culture on appropriate media, which is particularly useful where size or spore characteristics make identification difficult (the size and similarity of spores of *A. flavus* to other *Aspergillus* spp. make identification problematic). It operates by drawing air through an orifice at a rate of 16·5 L min^{-1} and deposits airborne particulates in still air in a microfuge tube at the base of the cyclone stream. A hot-wire anemometer (Model HHF51, Omega Engineering, Stamford, CT, USA) was used to ensure air was drawn at the correct rate. The size range of particles sampled by the Burkard has been characterized (Emberlin & Baboonian, 1995) and it samples particles in the size range of *A. flavus* spores (3–10 μm). Microfuge tubes were changed after each week of continuous operation. As conidia of *A. flavus* have been shown to survive a year in the field without loss of viability (Wicklow *et al*., 1993), it was assumed that the loss of propagule viability in the sample collected over a 7-day period by the Burkard traps was negligible. Although microfuge tubes were shaded from direct sunlight, exposure to light and storage for up to 7 days during sampling may have affected the viability of trapped spores of other species. Each sample was weighed, suspended in sterile distilled water, subjected to a dilution plate technique and plated on agar media, and incubated at 31°C for 5 days. Colony-forming units (CFU) of all fungi were counted on potato dextrose agar (PDA) and 5/2 agar (Cotty, 1989), and *A. flavus* CFU were counted on modified rose bengal agar (MRB) and 5/2 agar (Cotty, 1994b). PDA and 5/2 agar were amended with streptomycin (0·05 g L^{-1}) and chloramphenicol (0·05 g L^{-1}), added after autoclaving. The ratio CFU m^{-3} : air was calculated, and for comparisons between quantities of total fungi and *A. flavus* both enumerations were performed on 5/2 agar. After subculturing the *A. flavus* on 5/2 agar for 7 days at 31°C, all isolates were assigned to either the S or L strain by morphological criteria (Cotty, 1989). A total of 3307 *A. flavus* isolates (≈25 per sample) were subcultured and characterized. The proportion of *A. flavus* accounted for by the applied atoxigenic strain was estimated by vegetative compatibility group (VCG) analysis of the first 20 L-strain isolates, sampled every 4 weeks (720 isolates in total) as previously described (Bayman & Cotty, 1991; Cotty, 1994a).

Weather data

Weather data were obtained to investigate whether any parameters could be related to the production of CFU. The weather data were obtained from the Roll station, 32°44′40″ north, 113°57′40″ west of the Arizona Meteorological Network (AZMET, Soils, Water and Environmental Science Department, 429 Shantz Bldg #38, University of Arizona, Tucson, AZ 85721) which is located ≈68 km east of Yuma at an elevation of ≈91 m and ≈1·5 miles from the field site. Data were accessed at http://ag.arizona.edu/azmet/24html (files 2498ew.txt and 2499ew.txt). Details of the equipment and measurement protocols used at the Roll station may be found at this site. The mean weekly data for temperature (maximum, minimum and mean), rainfall and humidity were used to describe the weather conditions for the duration of the sampling period from May 1997 to March 1999.

Soil and surface samples

Samples were taken to assess the population composition in the four fields surrounding the samplers. In late June and October 1997, five replicate samples of soil, leaves, leaf litter and boll material were taken at random within 50 m of the cyclone sampler in the treated area. The top 2–3 mm of soil were sampled from a 2 m length of row (≈250 g). The lower and oldest leaves (100 g) were collected in June; in October 100 g of chemically defoliated leaves (following normal commercial practice) were collected from the ground. Boll samples (20 bolls per sample) were taken from the bottom half of the canopy with immature, unopened bolls sampled in June and fully mature, open bolls sampled in October.

In June and October 1998 samples were taken along a diagonal across each field, starting at that site's sampler at 25, 50, 150, 250 and 400 m, as described above.

Soil samples were mixed thoroughly; a subsample (5–20 g) was suspended in 50 mL sterile distilled water, agitated vigorously for 10 min, and subjected to the dilution plate technique on MRB agar. Leaf material (5–8 g in 200 mL),

leaf litter (5–17 g in 500 mL) and bolls/locules (10–25 g in 500 mL) were processed similarly. CFU of *A. flavus* were counted and CFU g^{-1} material calculated. Strain (S/L) composition and the proportion of the L strain in the applied VCG were estimated as for the cyclone samples.

Data analysis

Data were analysed using STATISTICA ver. 3·0 (Statsoft, Inc., Tulsa, OK, USA) and SAS ver. 8·0 (SAS Systems, Cary, NC, USA). Correlation analyses were used to relate total fungal CFU (minus *A. flavus*) with those of *A. flavus*. Total fungal CFU minus *A. flavus* was used in the analysis to avoid the influence of the *A. flavus* population *per se* on total CFU. Initially, correlation analysis was used to investigate the relationship between the weather variables (maximum, minimum and mean temperature, relative humidity, wind speed and rainfall) and the quantity of *A. flavus* sampled at the untreated and treated sites. A multiple regression analysis was used to investigate the association between environmental variables and the quantity of CFU sampled.

Autoregression (SAS PROC AUTOREG) analysis was performed to compare CFU counts from different media, sites (treated and untreated) and seasons (1997 and 1998). Autoregression estimates linear regression models for time-series data where the errors are autocorrelated. The Durbin–Watson statistic was used to diagnose autocorrelation using the ordinary least-squares estimation method and the data were reanalysed using the Yule–Walker estimation method. The total R^2 (coefficient of determination) for the full model was calculated to gauge goodness-of-fit of the parameter estimates.

Two different autoregression approaches were used to explore the data. In the first approach, the autoregressive error model [$y = a + bx + e$ where a, b and e (the autoregressed error) are parameter estimates] was fitted to the differences between the two sites (treated minus untreated) to test for significant differences in trends with time. Total fungal CFU, *A. flavus* CFU, and CFU from both media types were transformed by natural logs before taking the difference on each day. The percentage L and percentage of applied atoxigenic strain at the treated *vs* untreated sites was handled in the same way as the spore counts, but the percentages were not transformed before taking the differences and the few cases of 0% L were treated as missing observations. A significant effect of time indicates a difference in trend for these data. To compare the percentage L in 1997 *vs* 1998, the sites were autoregressed separately by year (it was not possible to match the data by days between years), and the data were transformed using arcsine, square-root transformation to stabilize the variance across days, and slope estimates between years were compared using the associated standard errors. The second autoregression approach was applied to data where no differences due to time were found using the first approach: overall differences between sites were tested by replacing time (day) by its deviation from the average, and thus the estimate of the intercept provides a measure of the difference between sites at the average of days, which gives a more powerful test of the underlying difference between sites (if the intercept is significantly different from zero they are different). The analysis was done to compare total fungal CFU and percentage L strain at the two sites, and CFU counts from both media types at the untreated site only.

The soil sample CFU counts were log-transformed, the proportion S strain and the proportion applied strain were arcsine square-root transformed and analysed using ANOVA (SAS PROC ANOVA). Tukey's HSD test was used to determine if means were significantly different.

Results

Characteristics of bioaerosol catches

The numbers of *A. flavus* CFU were counted on MRB. Autoregression analysis to compare trends with time (Table 2) showed the PDA *vs* 5/2 agar difference increased significantly ($P < 0{\cdot}001$) for the treated site, but did not change significantly ($P = 0{\cdot}207$) for the untreated site. However, the overall difference between PDA and 5/2 agar was significant ($P = 0{\cdot}007$) for the untreated site (Table 2). Thus total fungal CFU counts presented are from those counted on 5/2 agar, as it supported growth of more CFU. Although individual counts were not made, other fungi included species of *Aspergillus*, *Fusarium*, *Alternaria* and *Cladosporium*. The weight of material collected by the cyclone samplers varied from 0·001 to 1·0018 g, and exceeded 0·2 g on only two occasions. Total CFU was positively correlated to sample weight at the treated site ($r = 0{\cdot}4089$, df = 72, $P < 0{\cdot}05$), but not at the untreated site ($r = 0{\cdot}0528$, df = 71, not significant). However, the number of CFU of *A. flavus* sampled was positively correlated with sample weight at both the treated site ($r = 0{\cdot}2972$, df = 72, $P < 0{\cdot}05$) and the untreated site ($r = 0{\cdot}3207$, df = 71, $P < 0{\cdot}05$).

From May to December 1997 the total fungal CFU collected at the untreated site ranged from 17 to 667 m^{-3}, and at the treated site from 9 to 1277 m^{-3} (Fig. 2a). From January 1998 to March 1999 total fungal CFU ranged from 2 to 652 and from 2 to 412 m^{-3}, respectively, at the untreated and treated sites. Over the same periods counts of *A. flavus* ranged in 1997 from 1 to 416 and from 1 to 406 m^{-3}, and in 1998–99 from 0 to 117 and from 0 to 361 at the untreated and treated sites, respectively (Fig. 2b). Greatest numbers of both total fungal CFU and *A. flavus* CFU occurred between day 177 (1997) and day 43 (1998), and between day 191 (1998) and day 8 (1999). Very low numbers of all fungi were sampled between mid-January and May (sample weights at these times also tended to be lowest). *Aspergillus flavus* comprised 0–46% of the total cultured fungi at the untreated site and 0–51% at the treated site in 1997 (Fig. 3a,b); and 0–41% of the total cultured fungi at the untreated site and 0–44% at the treated site in 1998. At both sites *A. flavus* comprised a greater proportion of the total fungal population during the summer to autumn period.

Table 2 Autoregression[a] analyses to compare differences in numbers of fungal CFU, media types and between years using two Burkard samplers at different locations during 1997 and 1998 in Roll, Arizona

		Durbin–Watson[e]		Intercept			Day				
Comparison	Parameters	OLSE	Yule–Walker	Estimate	SE (*t* value)	*P* > *t*	Estimate	SE (*t* value)	*P* > *t*	MSE	$R^{b,g}$
Analyses[b] to compare trends with time between sites for numbers of fungal CFU, media and year											
Total fungi on PDA *vs* 5/2 media[c]	Treated site	1·53	2·03	−0·377	0·101 (−3·75)	<0·001	0·00077	0·00021 (3·75)	<0·001	0·235	0·28
	Untreated site	2·20	2·29	−0·308	0·135 (−2·28)	0·026	0·00034	0·00027 (1·27)	0·207	0·088	0·22
Treated *vs* untreated site	Total number of CFU	0·95	2·04	−0·157	1·036 (−0·15)	0·880	0·00084	0·00210 (0·40)	0·691	1·490	0·49
	CFU of *A. flavus*	1·52	1·94	1·199	0·788 (1·52)	0·133	−0·00387	0·00164 (−2·35)	0·022	2·690	0·39
	% L strain	0·81	2·05	18·638	15·96 (1·17)	0·248	−0·00928	0·0324 (−0·29)	0·776	382·31	0·50
	% L strain which is the applied atoxigenic strain	2·30	1·79	41·796	8·910 (4·69)	0·003	−0·05860	0·0199 (−2·95)	0·026	579·32	0·65
1997 *vs* 1998 over period day 147–360[d]	% L strain in 1997 (untreated)	1·14	1·77	−0·081	0·234 (−0·34)	0·736	0·00456	0·00093 (4·94)	<0·001	0·059	0·81
	% L strain in 1998 (untreated)	1·86	2·09	−0·137	0·201 (−0·68)	0·506	0·00427	0·00077 (5·52)	<0·001	0·074	0·67
	% L strain in 1997 (treated)	1·04	1·62	0·600	0·149 (4·03)	0·001	0·00246	0·00058 (4·23)	<0·001	0·021	0·76
	% L strain in 1998 (treated)	1·65	2·00	0·445	0·192 (2·32)	0·037	0·00256	0·00073 (3·49)	0·004	0·038	0·66
Analyses[f] to compare overall differences between sites at average day for numbers of fungal CFU and media types											
Total fungi on PDA *vs* 5/2 media[c]	Untreated site	2·19	2·29	−0·152	0·055 (−2·77)	0·007	0·00034	0·00027 (1·27)	0·21	0·09	0·22
Treated *vs* untreated site	Total number of CFU	0·95	2·04	0·225	0·394 (0·57)	0·57	0·00084	0·00210 (0·40)	0·69	1·49	0·49
	% L strain	0·81	2·05	14·408	6·123 (2·35)	0·02	−0·00928	0·03240 (−0·29)	0·78	382·31	0·50

[a]The autoregressive error model [$y = a + bx + e$, where *a* (intercept), *b* and *e* (the autoregressed error) are parameter estimates] was fitted to the differences between sites.

[b]The analysis compares trends with time (if the slope of the autoregression is significant there is a difference between sites due to day).

[c]Total fungal CFU, *A. flavus* CFU and CFU from both media types were log-transformed. The percentage L and percentage of applied atoxigenic strain at treated *vs* untreated sites were not transformed before taking the differences. The few cases of 0% L were treated as missing observations.

[d]To compare years, percentage L data were autoregressed separately by year after transformation using arcsine, square-root transformations.

[e]A Durbin–Watson statistic of ±2 suggests no autocorrelation. The OLSE (ordinary least-squares estimate) regresses *Y* on time (day) using least-square errors, which adversely affects parameter estimates and biases standard error estimates if data are autocorrelated, while the Yule–Walker fits an autoregressive model accounting for the autocorrelation.

[f]Differences between sites were tested by replacing time (day) by its deviation from the average – the estimate of the intercept (which is the estimate of the difference between sites) and then regressing. A difference between sites is indicated if the estimate of the intercept is significantly different from zero.

[g]R^2 = coefficient of determination.

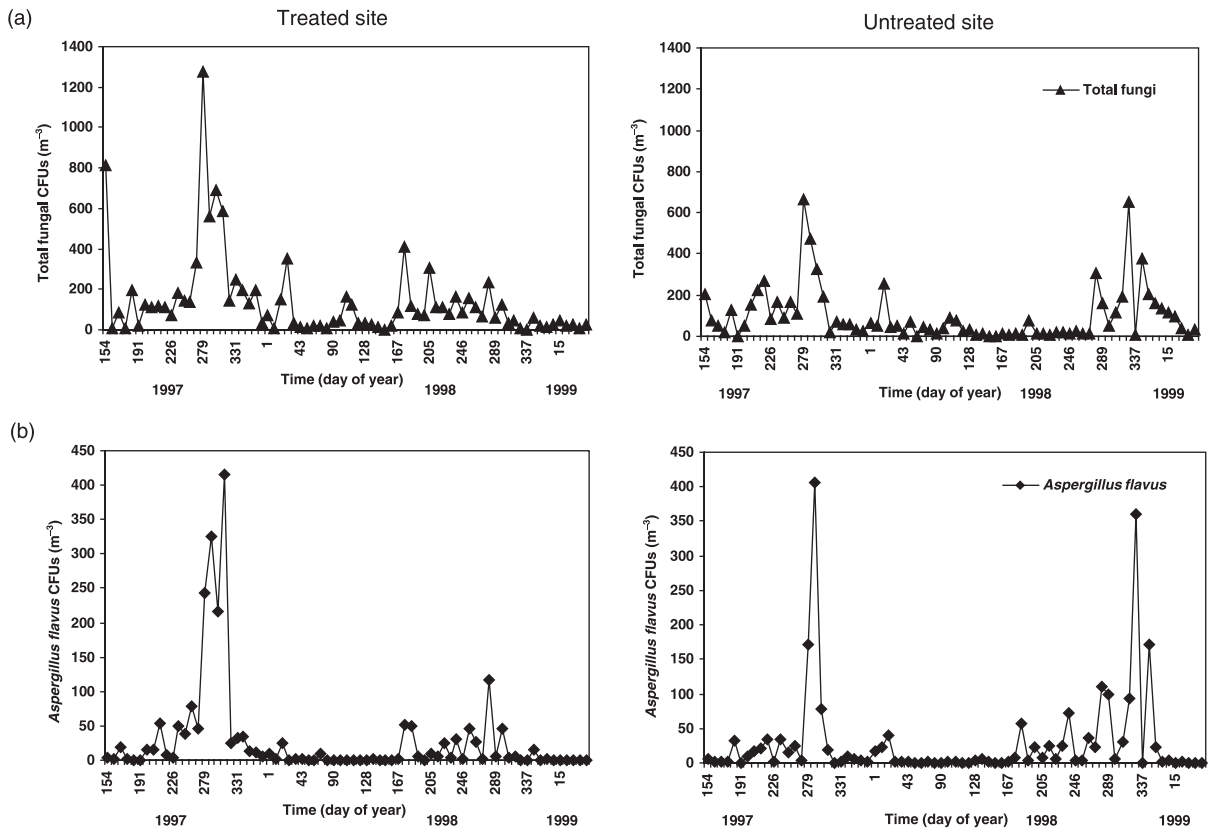

Figure 2 Quantity of (a) total fungi, and (b) *Aspergillus flavus* collected by Burkard cyclone samplers at a site treated with an atoxigenic strain of *A. flavus and* at an untreated site.

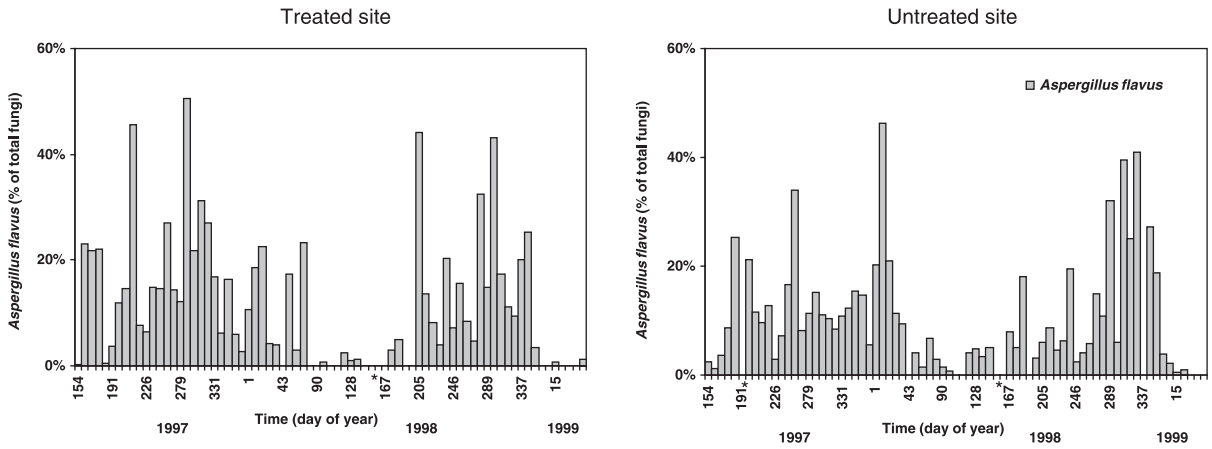

Figure 3 Proportion of total fungi consisting of *Aspergillus flavus* in the air sampled by Burkard cyclone samplers at a site treated with an atoxigenic strain of *A. flavus and* at an untreated site. *, Missing data points.

Autoregression analysis (Table 2) suggests no significant differences between the two sites in total fungal CFU over time, or in the overall number of CFU. However, autoregression analysis of CFU of *A. flavus* (Table 2) showed that the difference between the CFU of *A. flavus* at the treated and untreated sites was greater as time progressed ($P < 0{\cdot}022$) – the number of *A. flavus* CFU fell with time at the treated site. The treated site had relatively fewer CFU during the second year (Fig. 2b) while the untreated site had similar quantities in both years. Fields at the untreated site were under cabbage during much of 1997 and surrounded by cotton in March–October 1998 (cotton production favours *A. flavus*; Orum *et al.*, 1997), while the fields surrounding the sampler at the treated site

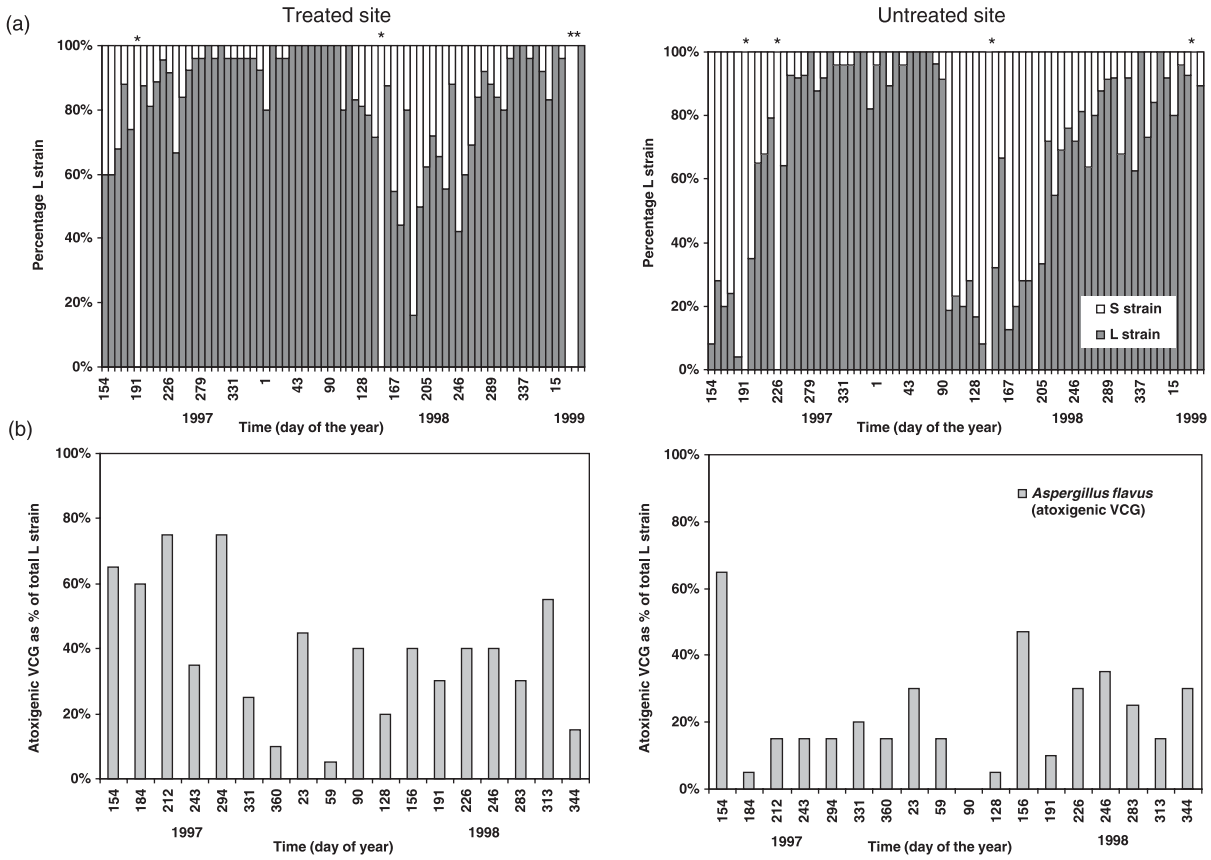

Figure 4 Characteristics of *Aspergillus flavus* communities sampled by Burkard cyclone samplers at a site treated with an atoxigenic vegetative compatibility group (VCG) and at an untreated site: (a) proportion of *A. flavus* consisting of L and S strains; (b) proportion of L-strain isolates belonging to the applied atoxigenic VCG. *, Missing data points.

were rotated from cotton in 1997 to wheat followed by lettuce in 1998 when counts of *A. flavus* were comparatively low. Correlation analysis showed a positive correlation between total fungal CFU and the number of *A. flavus* CFU at both the treated site ($r = 0{\cdot}5400$, 74 df, $P < 0{\cdot}05$) and the untreated site ($r = 0{\cdot}4200$, 73 df, $P < 0{\cdot}05$), indicating no major effect of treatment on the overall fungal population.

Both S- and L-strain isolates of *A. flavus* were detected at treated and untreated sites (Fig. 4a). Autoregression analysis (Table 2) showed no change in the percentage of the L strain with time between the two sites, but there was an overall significantly greater proportion of L strain ($P = 0{\cdot}02$) at the treated site (Table 2). Application of the atoxigenic L strain at the treated site was made in 1996 and 1997. Differences between sites were possibly established due to treatment in 1996, and in 1997 treatment was applied only 5 days after sampling commenced and the L strain applied probably established rapidly. The analysis suggests that the difference was maintained between sites throughout the sampling period, as there was no effect of time on the difference between sites.

There was distinct seasonality in incidences of S- and L-strain isolates, with the L strain most abundant from September to December At the treated site, 81·3 and 74·3% of the *A. flavus* was L strain between days 147 and 360 in 1997 and 1998, respectively, and at the untreated site over the same period 68·9 and 63·1%, respectively, was L strain. Autoregression analysis (Table 2) showed no difference between years in the percentage of L strain sampled for either site over these periods. Greatest quantities of S occurred between May and August in both years: 35·7–96·0% S (1997) and 28·0–100% S (1998) at the untreated site, and 4·5–100% S (1997) and 16·7–84·0% S (1998) at the treated site. The proportion of S at the treated site between May and August in 1997 (days 147–243, 30·5%) was greater than that present between September and December (days 148–365, 3·9%). Similar differences occurred in 1998 (38·6 *vs* 15·5%) at the treated site and in both years at the untreated site (54·7 *vs* 5·41% in 1997; 56·5 *vs* 17·2% in 1998). The applied atoxigenic VCG belongs to the L strain and accounted for 0–65% of the total L strain sampled at the untreated site, and 5–75% at the treated site (Fig. 4b). Autoregression analysis of the percentage of the applied atoxigenic VCG at the two sites showed that it was initially significantly more prevalent in the air at the treated site ($P = 0{\cdot}026$) than at the untreated site (Table 2), and that this difference decreased over time.

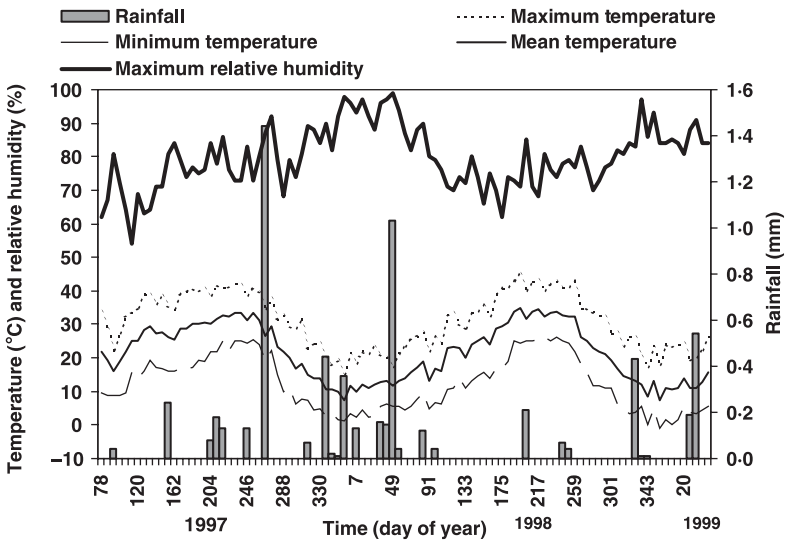

Figure 5 Weather data for Roll, AZ, USA from 1997 to 1999.

Relationship between CFU and weather data

In both 1997 and 1998, maximum daytime temperature (Fig. 5) was greatest between days 100 and 300 (30–45°C). In 1998 the period from days 78–155 was cool (accumulated mean degree-days >0°C = 246) compared with 1997 (accumulated mean degree-days >0°C = 286). Rainfall was infrequent and maximum relative humidity ranged from 54 to 100%. Average wind speed varied throughout the year, with the highest average wind speed occurring from day 100 to day 180 (≈3 m s^{-1}). The maximum wind speeds recorded exceeded 8 m s^{-1}, although for most of the time (71%) maximum wind speeds were <4 m s^{-1}. Wind blew from all directions throughout the year, and patterns were similar in the 2 years. There was a predominant wind from the south-west from April to September, with much of the wind blowing from the north-east from October to March

There was a positive correlation between the CFU of *A. flavus* at the treated and untreated sites ($r = 0{\cdot}6136$, $P < 0{\cdot}001$), and positive correlations between CFU of *A. flavus* at the treated and untreated sites and maximum temperature ($r = 0{\cdot}2394$, $P < 0{\cdot}05$; $r = 0{\cdot}2785$, $P < 0{\cdot}05$, respectively), minimum weekly temperature ($r = 0{\cdot}2803$, $P < 0{\cdot}05$; $r = 0{\cdot}2403$, $P < 0{\cdot}05$, respectively), and average temperature ($r = 0{\cdot}2424$, $P < 0{\cdot}05$; $r = 0{\cdot}2421$, $P < 0{\cdot}05$, respectively). Average soil temperature was correlated with CFU of *A. flavus* at the treated site only ($r = 0{\cdot}2337$, $P < 0{\cdot}05$). Multiple regression analyses returned unacceptably high coefficients of determination (R^2) between environmental variables that precluded a meaningful analysis.

Crop type and quantity of *A. flavus* sampled

The average number of CFU of *A. flavus* sampled with different crops varied considerably (Table 3). No crop consistently had the greatest average or maximum number of CFU sampled. Periods of fallow were characterized by high counts (mean of 77 and 39 CFU m^{-3} at the treated and untreated sites, respectively); these are periods of land preparation when considerable soil dust becomes airborne. The air around cotton crops (grown May–October) had a mean of 59 and 19 CFU m^{-3} at the treated and untreated sites, respectively; while the cabbage crop at the untreated site had an average of 45 CFU m^{-3}. The cabbage crop also had the greatest maximum number recorded (406 *A. flavus* CFU m^{-3}), although the reason for this is not known. Wheat

Table 3 Number of CFU of *Aspergillus flavus* sampled in the air surrounding different crops adjacent to the Burkard cyclone sampler at treated and untreated sites in the Mohawk Valley, Arizona

	Treated site			Untreated site		
	No. CFU m^{-3}			No. CFU m^{-3}		
Crop	Mean	Range	Cropping period	Mean	Range	Cropping period
Cotton	59	1–326	May–Oct	19	1–111	Mar–Oct
Cabbage				45	1–406	Aug 97–Jan 98
Lettuce	23	1–117	Aug–Nov			
Fallow	77	1–416	Jul, Nov	39	1–361	Feb, Jun, Jul, Nov
Wheat	6	1–51	Dec–Jun	20	1–171	Dec–Jun
Barley				3	–	May

Table 4 *Aspergillus flavus* communities in soil and on crop parts in fields treated with an atoxigenic strain of *A. flavus and* adjacent to a Burkard cyclone air sampler in 1997, in the Mohawk Valley, Arizona

Month	Substrate	CFU g^{-1}[a]	S strain (%)	Applied strain (%)[t]
June[c]	Soil	27 916 (3·98) a[d]	0 (1·00) l	92·7 (79·9) z
	Leaves[e]	5 748 (3·43) a	0 (1.00) l	100 (90·0) z
	Bolls	6 944 (3·39) a	0 (1·00) l	100 (90·0) z
	Mean	13 536 (3·51)	0 (1·00)	97·6 (86·6)
October	Soil	34 474 (4·14) a	0 (1·00) l	81·3 (72·8) z
	Leaves	272 461 (4·73) b	17·3 (24·6) m	79·3 (65·6) z
	Bolls	15 124 (3·72) a	0 (1·00) l	100 (90·0) z
	Mean	107 353 (4·28)	5·78 (8·19)	86·9 (76·1)
SED substrate type		0·58	0·29	8·2
SED sample time		0·34	0·17	4·8

[a]Figures in parentheses contain log transformations (CFU g^{-1}) and arc sine transformations (% S strain and % applied strain).
[b]Percentage of L-strain isolates assigned to applied VCG by vegetative compatibility analysis.
[c]Material was sampled in late June, treatment applied in early June.
[d]Means followed by the same letter are not significantly different ($P = 0{\cdot}05$) by Tukey's HSD test and refer to comparisons within columns.
[e]Leaf material: fresh leaves for late June sample; leaf litter resulting from chemical defoliation in October.

crops, grown primarily during the winter–spring period (December–June), when temperatures are lowest, had a mean of 6 and 20 CFU m^{-3} at the treated and untreated sites, respectively.

Soil, leaf and debris population counts

Quantities of *A. flavus* on cotton leaves, in soil and on bolls were compared from the treated site for June and October 1997 (Table 4). ANOVA indicated significant differences in the number of CFU between the two sample dates ($F = 5{\cdot}05$, $P = 0{\cdot}034$). The number of CFU on leaves collected in June and October was $5{\cdot}8 \times 10^3$ and $2{\cdot}7 \times 10^5$ CFU g^{-1}, respectively, and on bolls $6{\cdot}9 \times 10^3$ and $1{\cdot}5 \times 10^4$ CFU g^{-1}, respectively. There were significant differences in the prevalence of the S strain for both sample date and substrate types ($F = 2278$, $P < 0{\cdot}0001$; $F = 2191$, $P < 0{\cdot}0001$, respectively), with a greater proportion of the S strain on leaf litter (17·3%) and bolls (5·78%) in October. The S strain was not detected in soil from the treated site. Overall, a significantly higher proportion ($F = 4{\cdot}67$, $P = 0{\cdot}041$) of the applied strain was found in June (97·6%) compared with October (86·9%). In 1998 only soil was compared at the treated and untreated sites (Table 5). There were significant differences in the number of CFU between sites ($F = 16{\cdot}97$, $P < 0{\cdot}001$) and quadrats ($F = 8{\cdot}66$, $P < 0{\cdot}001$). The quantity of CFU g^{-1} soil at the treated site decreased from June (225) to October (58). However, at the untreated site CFU g^{-1} increased from 420 in June to $>1{\cdot}6 \times 10^4$ in October. The treated site in October was lettuce and the untreated site was cotton (Table 1). Site was the only factor significantly influencing the proportion of both S strain ($F = 87{\cdot}24$, $P < 0{\cdot}001$) and applied VCG ($F = 46{\cdot}49$, $P < 0{\cdot}001$). In June 1998 the S strain was more common at the untreated (70%) than at the treated site (14%). In October the incidence was 66 and 12%, respectively. In June the applied strain accounted for 85 and 13% of *A. flavus* at the treated and untreated sites, respectively, and in October the applied strain accounted for 65 and 36%, respectively.

Discussion

This study investigated the year-round population dynamics of airborne *A. flavus* and the effects of treatment with a biocontrol agent on the population structure. Environmental factors affecting the airborne *A. flavus* population around agricultural fields were assessed, and some indication was gained of the optimum times to treat fields with the atoxigenic strain. Although aspects of *A. flavus* dispersal in different crop systems and environments have been investigated (Morrow *et al*., 1964; Holtmeyer & Wallin, 1980, 1981; Lee *et al*., 1986; Olanya *et al*., 1997), this is the first year-round study of airborne *A. flavus* around cotton fields of south-western Arizona.

Dynamics of air-dispersed *A. flavus*

Peaks in total fungal CFU and those of *A. flavus* occurred between days 251 and 321 (September–November). 'Shoulder' periods with fewer CFU occurred before and after these dates. Lee *et al*. (1986) previously found large quantities of airborne *A. flavus* CFU associated with cotton production on individual sampling dates from July to September, but the quantities they observed were highly variable, perhaps associated with the brief sampling periods allowed by their Anderson sampler. In the current study, peak catches of microorganisms coincided with cotton harvest (September–November), stalk shredding, plough-down (incorporation of plant debris in the soil, mandated for insect control), and preparation of soils for winter crops, which create huge sources of organic debris for fungal growth. Reduced populations of *A. flavus* at the treated site during the second year (probably due to

Table 5 Quantity of *Aspergillus flavus* in soils of fields surrounding two Burkard cyclone air samplers in 1998 and incidence of both an atoxigenic vegetative compatibility group (VCG) of *A. flavus* (applied to the treated field in 1997) and the S strain of *A. flavus,* in the Mohawk Valley, Arizona

		CFU g^{-1}[a]		S strain (%)[b]		Applied VCG (%)[c]	
Month	Location[d]	Treated	Untreated	Treated	Untreated	Treated	Untreated
June	NE	491 (2·43) bc[e]	1 528 (2·67) b	12 (14) mno	63 (56) lmn	87 (77) xy	40 (36) y
	SE	122 (1·99) bcd	86 (1·79) bcd	0 (0) o	72 (58) lm	89 (73) xy	0 (0) z
	NW	208 (2·26) bcd	23 (1·04) cde	29 (29) mno	90 (81) l	100 (90) x	0 (0) z
	SW	80 (1·77) bcd	45 (1·47) bcd	13 (11) no	55 (48) lmno	66 (52) y	13 (11) yz
Treatment mean		225 (2·11)	420 (1·74)	14 (13)	70 (61)	85 (73)	13 (12)
Month mean		323 (1·92)		42 (37)		49 (42)	
October	NE	86 (1·80) bcd	198 (1·75) bcd	8 (11) no	60 (48) lmno	66 (58) y	50 (45) y
	SE	8 (0·75) de	29 (1·02) cde	20 (21) mno	49 (39) lmno	76 (67) xy	33 (30) y
	NW	130 (1·92) bcd	18 461 (4·03) a	17 (19) mno	67 (55) lmn	65 (57) y	30 (27) yz
	SW	8 (0·75) bcd	47 120 (4·54) a	3 (5) o	90 (78) l	55 (48) y	30 (27) yz
Treatment mean		58 (1·30)	16 452 (2·84)	12 (14)	66 (55)	65 (57)	36 (32)
Month mean		8255 (2·07)		39 (35)		51 (45)	
SED (month means)		0·129		4·6		6·1	
SED (treatment means)		0·182		6·5		8·6	
SED (bearing means)		0·364		13·0		17·3	

[a]Figures in parentheses contain log transformations (CFU g^{-1}) and arc sine transformations (% S strain and percentage Applied strain).
[b]Percentage of *A. flavus* isolates assigned to S strain by morphological criteria. All *A. flavus* isolates were assigned to either S or L strain (10).
[c]Percentage of L-strain isolates assigned to applied VCG by vegetative compatibility analysis (13).
[d]Location of field sampled in relation to Burkard cyclone sampler at that site.
[e]For each measured parameter, means followed by different letters are significantly different ($P = 0·05$) by Tukey's HSD test.

changes in cropping; Orum *et al*., 1997), the lack of a detectable difference in total fungal CFU between sites, and the positive correlation between total fungal and *A. flavus* CFU suggest there was no increase in *A. flavus* caused by application of the atoxigenic strain at the treated site. *Aspergillus flavus* was quantified on a selective medium (MRB) to ensure detection of the maximum number of *A. flavus* CFU. Other fungi were not grown on selective media, and were probably underestimated (fewer *A. flavus* CFU were detected on either 5/2 or PDA than on MRB), which agrees with previous reports on *A. flavus* isolation (Cotty, 1994b). There are probably substantial numbers of fungi that were either uncultivable on these media, competitively excluded, or inhibited by the incubation temperature (31°C), so the estimate of total fungi is likely to be rather low.

Dynamics of S and L strains and the applied biocontrol agent

The S strain comprised the highest proportion of total *A. flavus* during the summer period, while the L strain was most prevalent in winter and spring. The applied atoxigenic VCG belongs to the L strain, and the significantly greater proportion of the L strain at the treated site must be due in part to competitive exclusion of the S strain by the applied VCG (Cotty, 1994b). However, the quantity of L strain did not change with time, while the proportion of the applied strain decreased at the treated site, indicating that the applied L strain was being replaced by other L-strain types (most of which are toxigenic). The observation that the proportion of the L strain was higher overall at the treated site, and the applied atoxigenic L strain VCG was greater at the treated site than at the untreated site (albeit a decreasing proportion), suggests that the effect of treatment did not disappear after a single season (Cotty, 2000).

Although the quantity of *A. flavus* varied between season and site (there was less *A. flavus* at the treated site in 1998, while the quantity at the untreated site was comparable between 1997 and 1998), this is probably due to the surrounding cropping. Indications are that application of the atoxigenic VCG altered the population structure of *A. flavus* in the air, without increasing the total quantity of airborne *A. flavus*, and the relatively high incidence of the applied VCG in the air samples at the untreated site is probably due to dispersal from the treated site (distance between edge of treated area and sampler at the untreated site was 0·6 km). The wind was predominantly from the south-west from April to September, blowing towards the untreated site, and the wind speeds recorded (some >8 m s^{-1}) would have been sufficient to transport conidia between the sites. The ability of wind to disperse conidia at least several metres has already been demonstrated (Olanya *et al*., 1997). In the present study the atoxigenic VCG was applied to a 16 ha block in 1996 and to a 65 ha block in 1997, which constitutes a large source of inoculum for dispersal to the surrounding area. Indeed, the results suggest aerially dispersed conidia of *A. flavus* can travel hundreds of metres, which endorses an area-wide management strategy based on atoxigenic strains, as the cumulative effects of application would establish the atoxigenic VCG while minimizing ingress of aflatoxin-producing strains from other habitats. Area-wide application would also reduce contamination of all crops grown in the region, and reduce any health risk associated with

aflatoxin exposure via respiration of either aflatoxin-containing conidia or crop dust (Lee *et al*., 1983; Autrup *et al*., 1993). The current study illustrates the persistence of the applied atoxigenic strain 2 years after application, which corroborates earlier reports (Cotty, 2000). Long-term influences indicate that area-wide treatment strategies would benefit atoxigenic application, but further long-term work is needed to ascertain population changes and frequency of application.

Long-distance dispersal of *A. flavus* is also suggested by the discrepancy in the strain composition between air and soil samples at both treated and untreated sites. Large numbers of S-strain CFU were caught at the treated site in 1998 (days 160 to 253) when the soil analysis of the area around the sampler indicated a low incidence of the S strain. Either S-strain propagules were dispersed to the sampler from beyond the treated area (1–1·5 km), or there was local preferential dispersal of the S strain from soils during certain periods. Seasonal increases in S-strain incidence in soils of western Arizona in July/August have been described previously (Orum *et al*., 1997). Although the origin of the large proportion of the S strain remains unclear, the prevalence of the S strain in the air from May to December (peaking from July to September) spans the critical period of boll development and maturation. Strategies need to be refined in order to intervene in the movement of the S strain onto treated crops.

Factors affecting the aerial population of *A. flavus*

Cotton production in Arizona favours large populations of *A. flavus* (Orum *et al*., 1997), and the 1997 season was typical of the area. Despite a cool spring and early summer in 1998, CFU of *A. flavus* in the soil increased fourfold at the untreated site between June and October, but the quantity of *A. flavus* in the air was similar in both 1997 (when cabbage was grown) and 1998. The reason why there was not more airborne *A. flavus* in 1998 is unknown: perhaps the cooler temperature or the reduced amount of cotton in the area surrounding the untreated fields limited airborne CFU in 1998. CFU of *A. flavus* produced on cotton beyond the four fields surrounding the sampler may have contributed more in 1997 than in 1998. At the treated site in 1998 there were lower numbers of airborne *A. flavus*, and a fourfold reduction in the quantity of *A. flavus* in the soil between June (wheat planted) and October (lettuce planted), which was probably due to the change in cropping.

In Arizona, temperatures (>30°C) are frequently favourable for growth of *A. flavus* during cotton boll maturation (Ashworth *et al*., 1969a, 1969b; Ayerst, 1969; Marsh *et al*., 1973; Diener *et al*., 1987). A positive correlation was found between temperature and the quantity of *A. flavus* CFU collected. However, only a small proportion of the observed variation was explained by temperature. Other factors, including moisture, are required for growth of *A. flavus*. Although rainfall was infrequent, the regular irrigation in this region ensures adequate moisture, and there is ample dead plant material throughout the year for growth of *A. flavus*. Many anthropogenic activities disperse *A. flavus* into the air. Variation in these activities (cultivation, crop harvest, land levelling) was probably an important factor during the course of this study.

The date of application in relation to weather and crop development is an important criterion in maximizing the production of conidia and competitive exclusion of toxin-producing strains while minimizing the risk of product loss to predation or burial (Cotty & Bayman, 1993). Data presented here suggest that *A. flavus* increases in the air by early May, so earlier treatments are likely to be more effective provided temperature and moisture do not limit fungal metabolism and production of conidia. Application in the first week of May should be considered.

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